Nano-sized particles contained in tattoo inks: distribution and toxicity using ex-vivo human skin

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BACKGROUND

Tattoo inks contain metal **nanoparticles** (NPs < 100 nm) (Battistini et al., 2020; Bocca et al., 2017) that with their unique physico-chemical properties may result into novel mechanisms of metal uptake and cause skin or systemic toxicity in tattooed subjects.

AIM

We investigated distribution and potential toxic effects of nano-sized particles contained in tattoo inks in human skin explants from healthy donors.

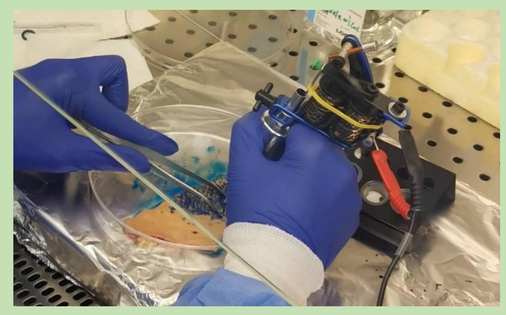
MATERIALS & METHODS

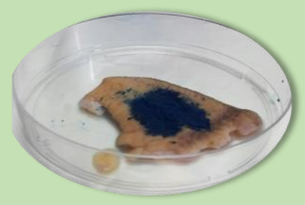
1) Three **tattoo inks** (blue, green and red) were firstly characterized by Single Particles Inductively Coupled Plasma Mass Spectrometry (**SP ICP-MS**) as reported in Bocca et al., 2020, and the metal NPs were observed (**Figure 1**).

2) The same inks were applied, using a **tattoo** machine as in the routine tattoo practice, into **human skin explants** obtained from healthy donors who signed an informed consent. Skin explants were incubated in keratinocyte basal medium (KBM) for 72 hours. Then, sections of 6 mm of diameter were obtained from each explant.

3) For the characterization by **SP ICP-MS explant** sections were subjected to **alkaline extraction** using 25% v/v TMAH, sonicated for 1 h, left at room temperature for 24 h and diluted with 0.1% v/v Triton X-100. Medium samples were water diluted.

4) For **histological and immunohistochemical analyses**, explant samples have been paraformaldehyde-fixed and paraffin-embedded. 4-m-thick sections were prepared, and specimens were deparaffinized, rehydrated, and processed. Hematoxylin and eosin staining was performed using standard procedures. Immunohistochemistry was performed using primary antibodies against p16, p53, interleukin (IL)-8 protein. Immunoreactivity was visualized with a peroxidase reaction using DAB (brown) or AEC (red) substrates





RESULTS & DISCUSSION

The **SP ICP-MS** analysis of the **medium and skin explants** (**Figure 1**) showed same average diameters for Cr_2O_3, CuO and ZnO (< 50 nm) in both the medium and explants, while smaller average diameters for Al_2O_3, Fe_2O_3 and TiO_2 in skin explants (49 nm, 37 nm and 153 nm, respectively) and larger in the medium (168 nm, 80 nm and 203 nm, respectively) (**Figure 2**).

Figure 1. Diameter of metal NPs in blue, green and red tattoo ink

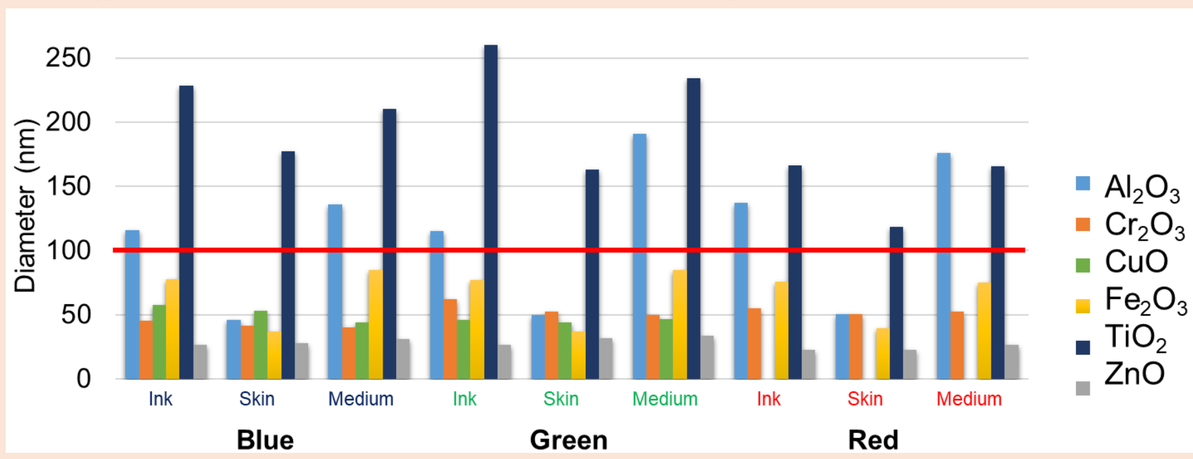
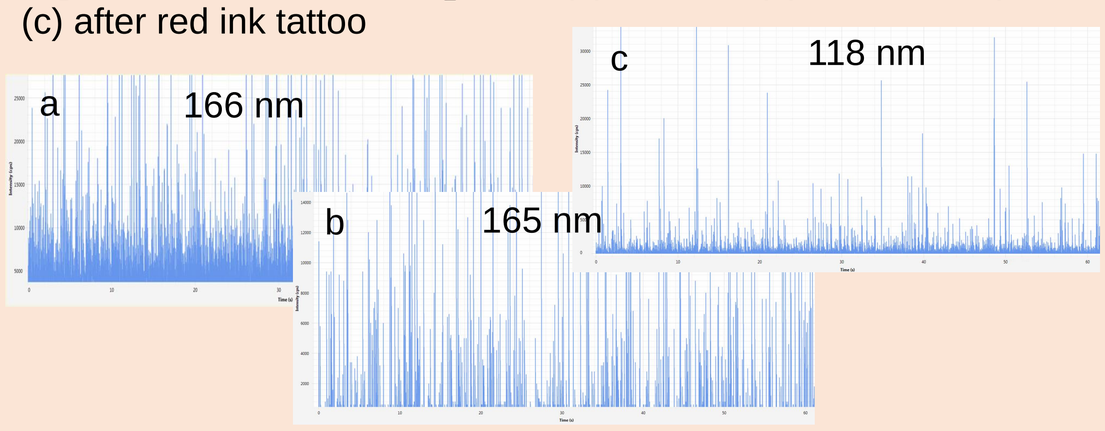


Figure 2. Raw data of TiO_2 in ink (a) medium (b) and skin explants (c) after red ink tattoo



The **histological analysis** showed the presence of pigments deep into the dermis and near dermal vessels suggesting the possibility of their systemic diffusion (**Figure 3**). Expression of the senescence/damage markers p16 (cytoplasm of epidermal and dermal cells; brown color) and p53 (cytoplasm of basal keratinocytes; red color) was observed, especially in the red ink tattoos. Expression by skin keratinocytes of the inflammatory protein IL-8 was visualized as well (**Figure 4**).

Figure 3. Histological analysis of green and red ink

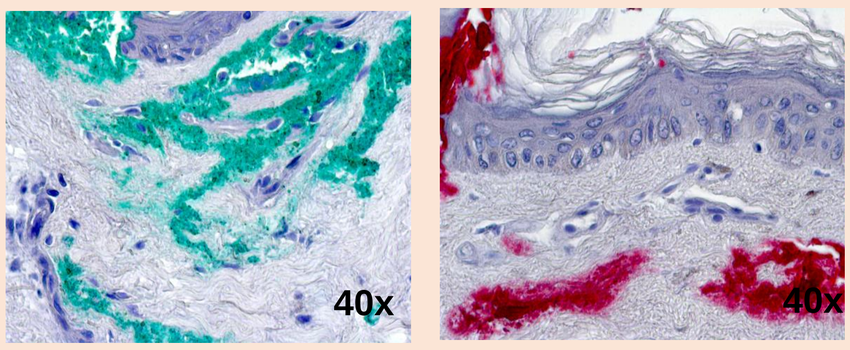
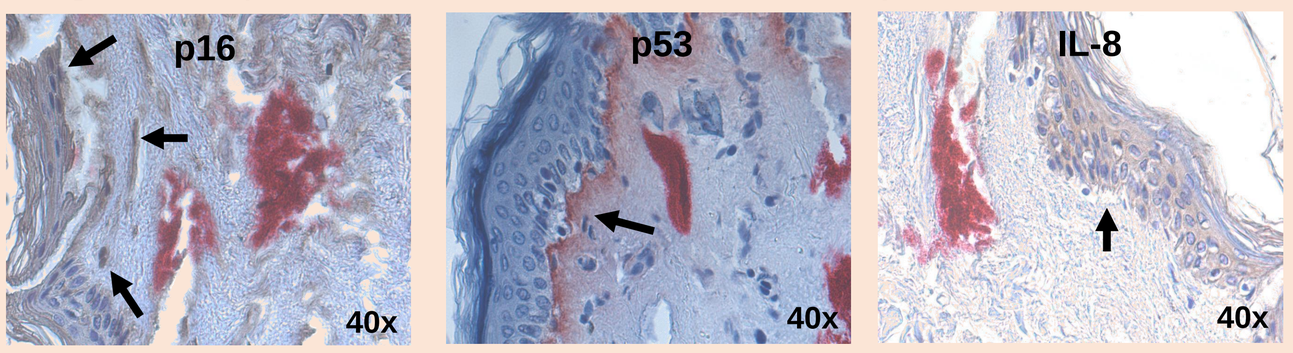


Figure 4. Representative immunohistochemical analyses of red ink tattoos



PRELIMINARY CONCLUSIONS

1) Overall, our results indicate that tattooing exposes humans to a mixture of NPs that differ in composition and size. Smaller size NPs tend to stay inside the skin, while larger ones are expelled.
2) The use of SP ICP-MS has proved to be a valid screening method (simple, fast and sensitive) for the presence of metallic NPs in human skin media and explants in order to characterize and evaluate the safety of tattoo inks.
3) The use of ex-vivo human explants, maintaining the original tissue architecture and not using animal derivatives (in compliance with the 3R principle) proved to be optimal for the study of skin toxicity. Furthermore, the results of this preliminary study allowed to set up the tattoo procedure on skin explants to be used in subsequent studies on the toxicity of the degradation products.

FUTURE OBJECTIVES

The future study (**ARTOO project**) will evaluate risks associated to tattoo laser removal using ex-vivo skin explants and the protocol here developed. In addition, the ARTOO project will measure the release of metals, nanoparticles and other hazardous molecules in blood and urine of tattooed individuals **before and after tattoo laser removal procedures.**

ACKNOWLEDGEMENTS

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